[0050] Where:

[0051] T_m represents the fraction of light allowed to pass through the epidermis without being absorbed by melanin.

[0052] T_{HbO} represents the fraction of light allowed to pass through the dermis to the collagen layer without being absorbed by oxyhemoglobin.

[0053] T_{Hb} represents the fraction of light allowed to pass through the dermis to the collagen layer without being absorbed by deoxyhemoglobin.

[0054] Thus, the Beers-Lambert formulation (i.e., the equation representing the principle that the degree of absorption of light varies exponentially with the thickness of the layer of the absorbing medium, its molar concentration and extinction coefficient) of equation 1 above is:

[0055] (2) $$\mathbf{A= -\ln(R_{tot}/ R_c) = 2\{I_E[M]\varepsilon_M + I_D[HbO]\varepsilon_{HbO} + I_D[Hb]\varepsilon_{Hb}\}}$$

[0056] Where:

[0057] **A** represents the absorbance at the site.

[0058] **I** represents the effective path length of the zone represented by the subscript.

[0059] **E, D** represents dermis and epidermis, respectively.

[0060] **[ ]** represents molar concentration.

[0061] **M, HbO, Hb** represent melanin, oxyhemoglobin and deoxyhemoglobin, respectively.

[0062] **ε** represents the molar extinction coefficient (unique for each wavelength).

[0063] Thus, it will be apparent that if blood is substantially prevented from entering a potential sampling site while an optical reading is taking place, the absorbance in equation 2 above is a function only of the melanin absorbance such that:

[0064] (3) $$\mathbf{A=-\ln(R_{tot}/ R_c)=2I_E[M]\varepsilon_M}$$ or

[0065] (4) $$\mathbf{\ln(R_{tot}) = \ln(R_c)-2I_E[M]\varepsilon_M = C,}$$

[0066] where **C** represents the melanin absorbance or background signal. Thus, the light absorbance resulting from hemoglobin can be represented by:

[0067] (5) $$\mathbf{\ln(R_{tot})=C-2\{ I_D[HbO]\varepsilon_{HbO} + I_D[Hb]\varepsilon_{Hb}\}}$$

[0068] Again, $\mathbf{R_{tot}}$ is the signal received by the photodetector. Thus, to obtain the background signal, a site having substantially no blood flow, i.e., a site where pressure is applied has been applied thereto to substantially prevent blood flow to the site, the absorbance due to hemoglobin only can be determined by first determining **C** from equation 4 above, where $\mathbf{R_{tot}}$ is the signal obtained from the first occluded optical measurement, and then solving for hemoglobin terms in equation 5 using $\mathbf{R_{tot}}$ from the second optical measurement where blood was not prevented from entering the site.

As such, since the molar extinction coefficients for both oxy and deoxygenated hemoglobin are known for all wavelengths in the visible and near infrared range (see for example O. W. Van Assendelft, *Spectrophotometry of Hemoglobin Derivatives*, Charles Thomas, pub., 1970), oxy and deoxygenated hemoglobin can both be determined by using more than one wavelength. Accordingly:

[0069] (6) $$\mathbf{I_D[HbO] = (C_1\text{-}ln(R_{tot})_1 - (C_2\text{-}ln(R_{tot})_2)(\varepsilon_{Hb1}/\varepsilon_{Hb2})/(\varepsilon_{HbO1}/\varepsilon_{Hb2})}$$

[0070] (7) $$\mathbf{I_D[HbO] = (C_1\text{-}ln(R_{tot})_1 - I_D[HbO](\varepsilon_{HbO1}/\varepsilon_{Hb1})}$$

[0071] Subscripts **1** and **2** represent wavelengths 1 and 2. In using the subject methods to characterize the hemoglobin of a potential site, the wavelengths are typically chosen so as to have very different extinction coefficients, i.e., wavelengths are usually chosen to make equations 6 and 7 as orthogonal as possible.

[0072] Accordingly, the first step in the subject methods to the characterize hemoglobin of a site is to determine the background signal at the site. By background is meant the absorbance of the site not related to hemoglobin, for example the absorbance related to melanin and the like. As such, light of two different wavelengths irradiates a potential site and the background signal is detected.

[0073] More specifically, wavelengths of light are chosen such that the molar extinction coefficient deltas of the oxy and deoxygenated hemoglobins are different for the different wavelengths chosen, i.e., as one molar extinction coefficient goes up the other molar extinction coefficient goes down, where such molar extinction coefficient deltas of oxy and deoxygenated hemoglobin are known in the art. Thus, to determine the background signal, the potential site is temporarily substantially occluded or rather blood is temporarily substantially stopped or prevented from entering the site, for example by pressing against the site, e.g., by pressing or applying pressure by the aperture of the

device described below onto the surface of the skin with enough force as to substantially stop blood flow to the site. In this way, the site is substantially devoid of any hemoglobin and thus any absorbance will be attributed to background or the absorbance of various chromophores at the site such as melanin. Once signal is detected from such an occluded potential site, the background value is then determined based upon the above described equations, typically automatically. More specifically, the signal detected by such a background determining method is communicated to a microprocessor, where such a microprocessor computes the background level or value of the site.

[0074] Following the background reading from the occluded site, a second reading at the site is taken. More specifically, light of two different wavelengths irradiates the site, where such wavelengths are chosen such that there is a large and opposite delta of the extinction coefficients of the two wavelengths. Once the signals from the two wavelengths are detected, the various components of hemoglobin can be determined from the above described equations, i.e., equations 6 and 7, typically automatically by a microprocessor as described above. In other words, oxygenated hemoglobin, deoxygenated hemoglobin and total hemoglobin (the sum of the oxygenated and deoxygenated hemoglobin components) can be determined, where such a determination can then be compared to a predetermined or cut-off value such that a total hemoglobin value and/or a hemoglobin ratio value, i.e., a ratio value defined by HbO/Hb, above the predetermined value is designated as a high hemoglobin value and a hemoglobin value below the predetermined value is designated as a low hemoglobin value. As noted above, alternatively, the values may be compared to other tested sites such that the best site among those tested is chosen.

[0075] Referring again to Figure 1, if a site has been characterized as having low flow, a further determination regarding total hemoglobin level will enable characterization of the site as having substantial vasculature (high total Hb) (6b of figure 1) or substantially devoid of vasculature, i.e., interstitial fluid (low total Hb) (6a of Figure 1). Once vasculature versus interstitial fluid or substantially no vasculature is determined, the site is then further characterized as being appropriate or not for a particular test (7 of Figure 1). In other words, if the particular test requires interstitial fluid, the potential sampling site will be determined to be appropriate if the total hemoglobin site is found to be low,